Docket No. K20606 US (C038435/0173527)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| In re Application of: | ) | |
| Hans-Peter HOHMANN *et al.* | ) | Examiner: Chih Min Kam |
| Serial No.: 10/681,086 | ) | Art Unit: 1656 |
| Filed: October 8, 2003 | ) | |
| For: **PROCESS FOR PRODUCING A TARGET FERMENTATION PRODUCT** | ) | |

**DECLARATION OF DR. NIGEL J. MOUNCEY UNDER 37 C.F.R. §1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Nigel J. Mouncey, a citizen of Great Britain, hereby declare as follows:

1. I studied microbiology at the University of Glasgow, Scotland from 1988 to 1992.

2. I received a doctorate degree in biochemistry in 1995 from the University of Sussex, Falmer, Brighton, E. Sussex, United Kingdom.

3. Since 1998, I have been employed at DSM Nutritional Products AG (previously Roche Vitamins Ltd. and Roche Vitamins AG), Kaiseraugst, Switzerland as a research scientist in molecular biology.

4. In 2006, I was appointed Senior Scientist in the area of strain development at DSM.

5. I am one of the inventors of the invention described and claimed in the above-captioned application directed to a PROCESS FOR PRODUCING A TARGET FERMENTATION PRODUCT.

6. The present application discloses and claims a process for producing a target fermentation product. One embodiment of the present invention is a process for decoupling production of a target fermentation product from biomass production in a fermentation medium. The process includes:

(a) providing a recombinantly produced microorganism from the genus Bacillus that has been engineered to contain a polynucleotide sequence which encodes biosynthetic enzymes for said target fermentation product,

(b) introducing a mutation causing a biotin auxotrophy into the microorganism to control biomass production and which does not compromise the ability of the microorganism to produce said target fermentation product, and

(c) supplying the medium with an unlimited amount of substrates required for the production of said target fermentation product and with a limited amount of biotin complementing the auxotrophy;

wherein said target fermentation product is riboflavin. (Specification at page 5, line 15 to page 6, line 14 and claim 23).

7. I am aware of and have reviewed the pending Office Action, Paper No. 20070803, that has issued with regard to the present application. It is my understanding that claims 23-26 and 32 have been rejected for lack of enablement and lack of written description under 35 U.S.C. § 112, first paragraph. (Paper No. 20070803 at 2-12).

8. In making the enablement rejection, the Examiner concluded that the claims were not commensurate in scope with the specification, and, therefore, that it would require undue experimentation to practice the claimed invention. (*Id.* at 2-8). The Examiner asserted that "the art does not disclose the mutation in the genes of the biotin biosynthesis that causes biotin auxotrphy" and "there is no [ ] structure/activity correlation for the mutated polynucleotides involved in biotin biosynthesis [in the specification], and the number of possible mutations in the biotin biosynthesis genes to be tested is virtually endless." (*Id.* at 5 and 8).

9. In making the written description rejection, the Examiner asserted that claims 23-26 and 32 "contain[ ] subject matter which was not described in specification

...." (*Id.* at 8). The Examiner further asserted that "the specification does not disclose a genus of variants for mutated polynucleotides that cause biotin auxotrophy in a transformed microorganism." (*Id.* at 10). The Examiner then concluded that:

> While the genes involved in biotin biosynthesis are known in the art, a convenient means may be used to introduce a mutation in the genes involved in biotin biosynthesis, and a screening method may be used to confirm a biotin auxotrophy, the specification does not disclose the structure/activity correlation for the mutated polynucleotides, and the number of possible mutations in the biotin biosynthesis genes to be tested is virtually endless. (*Id.* at 11).

10. In view of the foregoing, it is my understanding that the Examiner requires a showing of "structure/activity correlation for the mutated polynucleotides involved in biotin biosynthesis." (*Id.* at 8 and 11).

11. In order to help provide this showing and to help demonstrate that a person skilled in the art would readily be able to make and use the full scope of the claimed invention and that Applicants were in possession of the claimed invention as of the filing date, I submit a copy of Bower *et al.*, "Cloning, Sequencing, and Characterization of the *Bacillus subtilis* Biotin Biosynthetic Operon," J. Bact., Vol. 178, No. 14, pp. 4122-4130 (1996) ("Bower") (Exhibit 1). Bower discloses a cloned and sequenced "10-kb region of the *Bacillus subtilus* genome that contains genes involved in biotin biosynthesis." (Abstract). Bower discloses that the "*bio* genes of *B. subtilis* are located in a single operon and that genes with similarity to *bioW*, *bioA*, *bioF*, *bioD*, and *bioB* are found in this operon." (Page 4122). Bower also discloses the construction of

various *Bacillus subtilis* mutants which require biotin for their normal growth. (Pages 4127-28). Bower further discloses insertion and deletion mutations within the *Bacillus subtilis* bio operon and flanking DNA. (Page 4127 and Figure 4 (reproduced below)). Bower further discloses the location of these mutations. (Pages 4127-28 and Table 4).

12. Mutations are diagrammed in Figure 4:

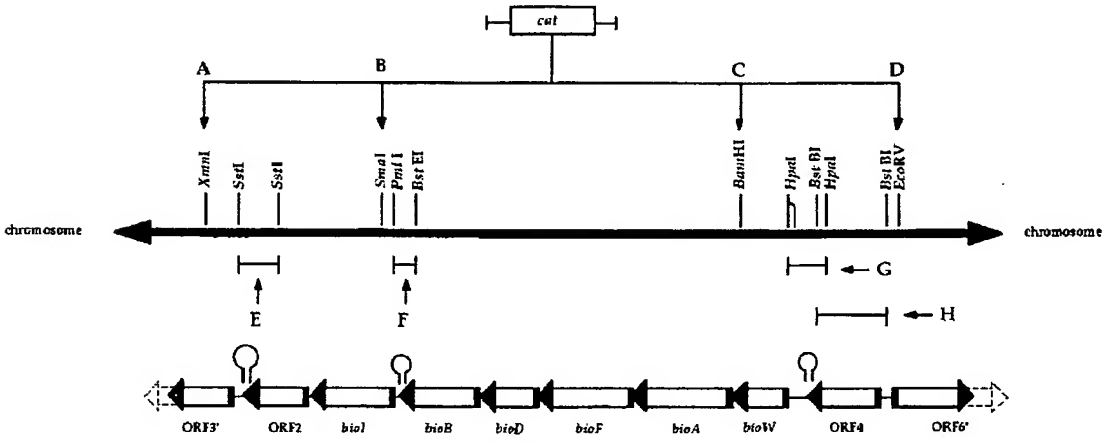


Fig. 4 Locations of *cat*-containing insertions and deletions within the *B. subtilis bio* operon and flanking DNA. As described in Materials and Methods, in vivo mutations of the *bio* genes and flanking open reading frames were generated either by inserting a 1.5-kb *cat*-containing cassette into the indicated restriction site (A, *Xmn*I; B, *Sma*I; C, *Bam*HI; or D, *Eco*RV) or by replacing the indicated region with the *cat* cassette (E, replacement of a 606-bp *Sst*I fragment; F, replacement of a 260-bp *Pml*I-*Bsp*EI fragment; G, replacement of three adjoining *Hpa*I fragments totaling 313 bp; H, replacement of a 966-bp *Bst*BI fragment). Not all restriction sites are shown. *B. subtilis* strains containing these mutations were examined for their biotin phenotypes. And the results are tabulated in Table 4.

13. Table 4 of Bower, reproduced below, discloses the characterization of insertion and deletion derivatives of the biotin operon:

TABLE 4. Characterization of insertion and deletion derivatives of the biotin operon

| Biotin operon derivative (mutation) and *cat* gene orientation[a] | Biotin phenotype[b] | Growth on[c]: | | |
|---|---|---|---|---|
| | | Minimal medium[d] | DTB[e] | Pimelic acid[f] |
| Wild-type *bio* operon | + | + | + | + |
| A (Ω*orf3*) | | | | |
| R | + | + | | |
| L | + | + | | |
| B (Ω*bioI*) | | | | |
| R | +/− | +/− | + | + |
| L | +/− | | | + |
| C (Ω*bioW*) | | | | |
| R | − | − | − | − |
| L | − | | +/− | |
| D (Ω*orf6*) | | | | |
| R | + | + | | |
| L | + | + | | |
| E (Δ*orf2*) | | | | |
| R | + | + | | |
| F (Δ*bioB*) | | | | |
| R | − | | − | |
| G ($\Delta P_{bio}$) | | | | |
| R | − | | − | − |
| L | +[g] | | + | +[g] |
| H (Δ*orf4*) | | | | |
| L | + | + | | |

[a] See Fig. 4 for a map of *cat* insertions within the biotin operon. Insertion derivatives having the *cat* gene in either orientation were obtained; R (right) and L (left) identify the transcriptional orientation of the inserted *cat* gene when the *bio* operon is oriented as shown in Fig. 4.

[b] Biotin phenotype determined by patching bacteria on biotin-free agar plates. +, biotin prototroph; +/−, biotin bradytroph; −, biotin auxotroph.

[c] +, prototrophic; +/−, bradytrophic; −, auxotrophic.

[d] Growth of bacteria on Spizizen's minimal medium agar plates.

[e] Growth of bacteria on biotin-free agar plates containing 33 μg of DTB per liter.

[f] Growth of bacteria on biotin-free agar plates containing 33 μg of pimelic acid per ml.

[g] Appearance at a frequency of 0.1% of $Bio^+$ bacteria in which biotin synthesis is inducible by chloramphenicol.

14. In addition, I submit a copy of Sasaki *et al.*, "Genetic Analysis of an Incomplete *bio* Operon in a Biotin Auxotrophic Strain of *Bacillus subtilis* Natto OK2," Biosci. Biotechnol. Biochem., Vol. 68, No. 3, pp. 739-742 (2004) ("Sasaki") (Exhibit 2). Sasaki "describe[s] the genetic analysis of the *bio* operon of the biotin auxotrophic *Bacillus subtilis* natto OK2 strain." (Abstract and Page 739). Sasaki discloses the introduction of mutants into the biotin operon leading to an auxotrophy. (Pages 740-41). For example, Sasaki discloses a BioW gene mutation, which results in an opal stop codon. (Page 740). Sasaki further discloses a deletion mutation in the BioF gene. (*Id.*). Sasaki also discloses the location of these mutations. (*Id.* and Figure 1).

15. Those documents confirm that generating biotin auxotrophic mutants as described and claimed in the above-captioned application was within the skill in the art. In my opinion, the level of knowledge and skill in this art is high - one having skill in the art typically being at a post-doctoral level or higher. Indeed, the Examiner has confirmed this. (Paper No. 20070803 at 5). In my opinion, the mutations disclosed in Bower for *Bacillus subtilis* together with the known sequence of the whole bio-operon, the disclosure of the biotin mutant in the present invention, and the disclosure of a method for confirming biotin auxotrophy in a mutant is more than enough to provide a person skilled in this art sufficient guidance to generate bio-auxotrophic mutants via the process claimed.

16. To demonstrate that the claimed process is enabled, I note that the genes involved in biotin biosynthesis are well known to those skilled in the art. (Sasaki, Page

739). In this regard, the specification discloses that the mutation causing auxotrophic growth may be introduced using any convenient means, such as for example by "chemical and UV mutagenesis followed by screening or selection for a desired phenotype." (Specification, p. 8, lns. 16-19). Simple screens for confirming an auxotrophy are likewise disclosed in the specification:

> A microorganism that is an auxotroph for biotin is unable to grow without supplementation with biotin, *i.e.*, the substrate complementing the auxotroph. (*Id.*, p. 12, lns. 18-20).

The specification also discloses a specific exemplification of a process for decoupling production of riboflavin from biomass production with biotin auxotrophy, including a description of how to make a specific biotin auxotroph. (*See*, *e.g.*, Specification, pp. 15-18; Examples 1-3; and Figs. 1-4).

17. Based on my knowledge and experience, and in view of the statements presented herein, it is my opinion that the Examiner's concern that a person would not know how to choose a proper mutated polynucleotide that causes biotin auxotrophy from a large number of mutated sequences is misplaced. (*See*, Paper No. 20070803 at 8). As one skilled in this art is well aware, the disclosure of a method for identifying a desired characteristic, here a biotin auxotrophy, is what is important. Screening large numbers of mutants is simply a matter of routine lab work that does not require inordinate skill. Moreover, in this art there is no need to identify structure/activity correlations. Because the bio operon was well known and characterized, manipulating different genes within the operon to suit one's purpose was well within the skill of the art when the present application was filed. Thus, in my opinion, once the desirability of

making a particular mutation (*e.g.*, a biotin mutant) became known, and once how to identify and screen for the particular mutation became known, and once it became known how such a mutation could be used through the present application (*see, e.g.*, Specification at page 12, lines 14-22 and Figures 3-4), it was - and is - within the skill of the art to make any number of such mutants.

18. The specification also describes how to make mutations that may lead to auxotrophs (*see, e.g.,* Specification, p. 8, lns. 16-19), simple assays for confirming an auxotrophy (*Id.*, p. 12, lns. 18-20), and exemplification of the specific biotin auxotroph (*see, e.g.*, Specification, pp. 15-18; Examples 1-3; and Figs. 1-4).

19. The specification also describes how to identify *Bacillus* strains that would fall within the scope of the process claimed. The specification describes, for example, at page 11, lines 17-20 the production rate of a microorganism carrying an auxotrophy; at page 12, lines 18-20 the specification describes that a microorganism that is an auxotroph for biotin is unable to grow without supplementation with biotin (if the strain is a biotin-auxotroph); and at page 14, lines 8-11 the specification describes an increase of at least 0.1% in the yield of the target fermentation product after engineering of the host microorganism.

20. Examples 1-3 of specification also describe construction of biotin auxotrophic *Bacillus* mutants, continuous culture fermentations, and biomass and riboflavin production in coupled and decoupled processes. (*See* Examples 1-3).

Furthermore, I note that the *B. subtilis* strain RB50 and plasmid pRF69 have been deposited. (*See, e.g.,* page 8, lines 10-15).

21. Based on my knowledge and experience, and in view of the statements presented herein, it is my opinion that the information disclosed within the specification, the knowledge of the structure of the biotin operon and the genes contained therein, and the deposited materials provide adequate description for one skilled in the art to envision the genus of mutants called for by the claim and to use them in the claimed process. In addition, it is my opinion that a person skilled in the art at the time the present invention was made and who was familiar with the knowledge in the art, including the cited Bower and Sasaki articles, would be able to make and use the claimed invention.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 7th February 2008

Nigel J. Mouncey

Dr. Nigel J. Mouncey